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(54) Title: DETECTING GENETIC PREDISPOSITION TO PERIODONTAL DISEASE

(57) Abstract

A method and kit for the identification of a patient's genetic polymorphism pattern associated with increased periodontal disease severity is disclosed. The kit includes DNA sample collecting means and means for determining a genetic polymorphism pattern which is then compared to control samples to determine a patient's susceptibility to severe periodontal disease.

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DETECTING GENETIC PREDISPOSITION TO PERIODONTAL DISEASE

BACKGROUND OF THE INVENTION

TECHNICAL FIELD

This invention relates to a method of detecting a predisposition for severity of periodontal disease.

BACKGROUND ART

Periodontal disease is a disease of the hard and soft tissues that support the teeth and is initiated by oral bacteria. Gingivitis is an early stage of the periodontal disease where the gums may become red, swollen and bleed easily. Gingivitis is usually painless and, if not treated, can advance to periodontitis, which may be classified by the magnitude of tissue destruction as mild, moderate, or severe. Periodontitis is primarily a disease of adults and is usually not detectable until after the age of 35.

Bacteria that are present in dental plaque initiate periodontal disease. Toxins produced by the bacteria in the plaque activate the body's inflammatory and other immune mechanisms which ultimately leads to the destruction of the bone and gum tissue that support the teeth. As the disease progresses, the gums pull away from the teeth and periodontal pockets are formed which provide a protected environment for the bacteria, thereby causing the cycle to continue. However, some sites do not continue to be active. United States patent 5,328,829 discloses a method for determination of active periodontal disease sites within the oral cavity by measuring interleukin IL-1β at the site. Smoking has been associated with an increased prevalence and severity

of periodontitis. However, a significant number of individuals with periodontitis have never smoked.

For the past 15 years, there has been evidence that certain forms of periodontitis that affect young children and teenagers are genetically determined. These diseases, which are of extremely low prevalence in the population, produce severe periodontitis in some individuals before the age of puberty and in other individuals between puberty and age 18. The genetic factors that were identified in those cases involved very overt biologic mechanisms that most likely would predispose the individual to multiple health problems. To date, efforts to find the same types of genetic factors in adult forms of periodontitis have not been successful.

In spite of the above failures, new evidence emerged beginning in 1990 from studies of identical twins that indicated that genetics play a significant role in the clinical presentation of disease in adult forms of periodontitis (Michalowicz et al., 1991). While the twin studies indicated that there was a genetic component, it was not identified. It would be useful to determine patients who are susceptible to severe adult periodontitis.

Genetic testing is now possible (see United States Patents 4,582,788 and 5,110,920) for diseases associated with or caused by one to two genes, once the genes are identified, to determine the risk of a person carrying a given gene for the disease (see for example United States Patents 4,801,531, 4,666,828 and 5,268,267).

As with any infection, once initiated, inflammatory and other immune mechanisms of the body come into play (see United States Patent 5,328,829, column 1, for a review). In general, research on inflammatory markers has had very limited success at differentiating

periodontitis disease severity and there have been limited and unsuccessful efforts directed to the genetic aspects of the inflammatory response of periodontal disease. Genetic variation at the multiple loci controlling the inflammatory and other immune responses in selected diseases with inflammatory components has been a factor in determining susceptibility to, or severity of, disease. Therefore, it was an objective of the present invention to determine if genetic factors that are associated with inflammatory and other immune responses are correlated with periodontal disease severity. If so, it would be useful to identify the genetic factors and thereby identify patients who are susceptible to severe forms of adult periodontal disease.

SUMMARY OF THE INVENTION

According to the present invention a method for predicting increased periodontal disease severity is disclosed. The method includes the steps of isolating DNA from a patient and determining the DNA polymorphism pattern of the genes that code for IL-1α and IL-1β. The identified pattern is compared to controls of known disease severity thereby identifying patients expressing a genetic polymorphism pattern associated with increased periodontal disease severity. Patients so identified can then be treated more aggressively in the early stages of periodontal disease to prevent the occurrence of severe disease.

The present invention further discloses a kit for the identification of a patient's genetic polymorphism pattern associated with increased periodontal disease severity. The kit includes DNA sample collecting means and means for determining a genetic polymorphism pattern, which is then compared to

control samples to determine a patient's susceptibility to severe periodontal disease.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

According to the present invention, patients with or without overt disease are identified as having a genetic predisposition for severe periodontal disease by detecting the presence of a DNA polymorphism in the gene sequence for interleukins IL-1α and IL-1β. Severe periodontal disease is defined as set forth in the example herein below. Briefly, severe disease is defined as patients presenting with a history of ≥10 interproximal sites that measure ≥7 mm, with pocket depth (PD) of ≥7 mm occurring on at least eight teeth. In addition, clinical attachment (CAL) measured ≥5 mm on ≥11 sites is seen. The definition further requires that full mouth radiographs taken within the last three years shows ≥7 interproximal sites with ≥50% bone loss on radiographs with a total mouth mean bone loss greater than 30%.

The alleles associated with severe disease were identified as IL-1A allele 2 together with IL-1B (*Taq*I) allele 2. It was determined that the Odds Ratio (OR) for severe periodontitis is 4.3 for patients carrying at least one copy of IL-1A allele 2 and IL-1B (*Taq*I) allele 2 among nonsmokers. In a population of smokers and nonsmokers the OR for a smoker or patients carrying at least one copy of IL-1A allele 2 and IL-1B (*Taq*I) allele 2 is 10.06 for having severe disease.

Further, according to the present invention, a kit for the identification of a patient's genetic polymorphism pattern associated with increased periodontal disease severity is disclosed. The kit includes DNA sample collecting means and means for determining a genetic polymorphism pattern for IL-1A and IL-1B, which pattern is then compared to control samples

to determine a patient's susceptibility to severe periodontal disease.

The DNA sample is obtained from blood or tissue samples. In a preferred embodiment, the DNA will be obtained from blood cells obtained from a finger prick of the patient with the blood collected on absorbent paper. In a further preferred embodiment, the blood will be collected on an AmpliCard™ (University of Sheffield, Department of Medicine and Pharmacology, Royal Hallamshire Hospital, Sheffield, England S10 2JF). The DNA is then isolated from the dried blood spots and then target sequences amplified using the polymerase chain reaction (PCR). Oligonucleotide DNA primers that target the specific polymorphic DNA region within the genes of interest are prepared so that in the PCR reaction amplification of the target sequences is achieved. This embodiment has the advantage of requiring only a small amount of blood and avoids the necessity for venipuncture or a tissue biopsy. However, other means for collecting DNA and determining polymorphism patterns as known in the art can be used.

The amplified DNA sequences from the template DNA are then analyzed using restriction enzymes to determine the genetic polymorphisms present in the amplified sequences and thereby provide a genetic polymorphism profile of the patient.

Some diseases have prominent inflammatory and other immune components. One of the primary components of the inflammatory and other immune responses is cytokine production. Cytokines are peptide/protein immunomodulators that are produced by activated immune cells including thymus-derived T lymphocytes (T-cells), B lymphocytes and monocyte/macrophages. The cytokines include interleukins (IL-1 through IL-15), colony stimulating factors (CSFs) for granulocytes and/or macrophages (CSF-G, CSF-M, CSF-GM), tumor necrosis

factors (TNFs α & β), and interferons (IFN α, β & γ). The basic activity of IL-1 includes the combined activities of IL-1α, IL-1β and IL-1 receptor antagonist (IL-1ra). (For a review, see Duff, 1993; and *Basic and Clinical Immunology*, 8th Ed., 1994, Stites, Terr & Parslow, editors, Chapter 9, pgs. 105-123.). United States patent 5,328,829 found IL-1β at active sites in periodontal disease but did not report any correlation with disease state. Association of a single cytokine polymorphism and disease states have been found as, for example, in Systemic Lupus Erythematosus, Ulcerative Colitis and Juvenile rheumatoid arthritis (Mansfield et al., 1994; Verjans et al., 1992; Blakemore et al., 1994; McGuire et al., 1994; McDowell et al., 1995).

Specific polymorphisms in DNA sequences coding for cytokines IL-1α and IL-1β were found to be associated with severe periodontal disease. The polymorphisms are as follows:

IL-1A: (chromosome 2 at 2q12-14)

The alleles of a bi-allelic polymorphism of a single base variation (C/T) at -889 are identified by allele-specific cleavage using a restriction enzyme. The gene is designated IL-1A while the product (cytokine) is designated IL-1α. Allele 1 is C and allele 2 is T at base -889. The full restriction enzyme recognition site is created by introducing a partial site by mutation in the PCR reaction with a modified primer sequence. The site is completed by the sequence of one of the alleles of the polymorphism. After restriction enzyme digestion of the products of the PCR reaction, the DNA is separated electrophoretically by size.

From this gel (or a southern blot of it probed with a radioactive internal DNA sequence) the alleles of the polymorphism are identified. The uncut fragment

(larger) is the rarer allele in Northern European populations.

IL-1B: (chromosome 2; 2q12-14)

Two bi-allelic polymorphisms can be typed in two different PCR products using allele-specific cleavage at naturally-occurring sites in the alleles. Allele identification is by size of fragment after restriction digestion and separation in an agarose gel. The gene is designated IL-1B while the product (cytokine) is designated IL-1β. The sites are single base variations (C/T) at -511 (referred to as IL-1B (*Ava*I)) and at +3953 (referred to as IL-1B (*Taq*I)) and are identified by allele-specific cleavage using restriction enzymes. For each polymorphism allele 1 is C and allele 2 is T.

The patient's cytokine polymorphism profile, i.e., allelic distribution, is then compared to controls. The controls are from patients who are periodontally healthy and from adult nonsevere periodontitis patients and adult severe periodontitis patients. That is, the patient's profile is compared to healthy people and patients with periodontal disease of different severity according to consensus clinical criteria and the match determines the predisposition towards periodontal disease. In one embodiment, controls are provided that are ethnically matched to accommodate genetic variations within subpopulations.

An odds ratio (approximate relative risk) is derived to test the association between allelic polymorphism pattern (genotype) at these specific loci and development of disease and/or its severity. This provides predictive information that will be used in the clinical management of periodontal disease.

The above discussion provides a factual basis for a kit for the identification of a patient's genetic polymorphism pattern associated with increased

periodontal disease severity. The identification of those at risk for severe disease allows preventive measures to be initiated prior to disease onset. Further, those patients who have two risk factors, smoking and the susceptible genotype, can be particularly monitored since their risk of severe disease is extremely high. The methods used with and the utility of the present invention can be shown by the following example.

EXAMPLE

POLYMORPHISM DETERMINATION AND DISEASE ASSOCIATION

General Methods

Reactions and manipulations involving DNA techniques, unless stated otherwise, were performed as described in Sambrook et al., 1989, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, incorporated herein by reference. Methodology as set forth in United States patents 4,666,828; 4,801,531; and 5,272,057 and McDowell et al., 1995 are also used unless stated otherwise.

Enzymes used in PCR were from GIBCO BRL, thermocyclers were either Perkin-Elmer or Biometra. Restriction enzymes *Nco*I and *Taq*I were from Promega (US). Restriction enzymes *Ava*I and *Bsu*36I were from NEB (US).

Patient Selection and Disease Classification

Genetic polymorphisms associated with periodontal disease in adults was determined using the protocol of McDowell et al. (1995). Because of the masking effect of smoking, genetic factors associated with severe disease were determined in nonsmokers. A group of otherwise healthy adults were screened at a dental clinic for the presence of periodontal disease. The study included primarily individuals of Northern European descent. Each patient was screened for the

absence of disease or, if the disease was present, its degree in each of four parameters. The four variables of interest are clinical attachment loss (CAL), pocket depth, gingivitis and interproximal bone loss. A blood sample is taken, DNA isolated and the genetic polymorphism at IL-1A and IL-1B genetic loci determined. In addition, a dental history of each patient was obtained including specific questions on family history of diabetes, cardiovascular disease or early tooth loss as well as whether they were smokers.

In order to determine periodontal disease status, each patient underwent an examination including a full mouth measurement of pocket depth (PD), recession (R), plaque (Pl) and bleeding on probing (BOP). Clinical attachment loss (CAL) is computed from pocket depth and recession. Radiographs assess bone loss. Based on these measurements, the patient was classified as either healthy, mild to moderate periodontitis or severe periodontitis.

All clinical variables were calculated on six surfaces (distal buccal, buccal, mesial buccal, mesial lingual, lingual and distal lingual) on each tooth (excluding third molars) for up to 168 sites. All radiographic variables were calculated on two surfaces on each tooth for up to 56 sites.

Disease severity classification is as follows:

Periodontally Healthy: Patients presented with all pocket depths ≤4mm, unlimited facial CAL, interproximal CAL of ≤2mm and <15% radiographic bone loss. Unlimited plaque and gingival inflammation and recession may be present.

Mild to Moderate Periodontitis: No history of disease onset prior to age 35. Patients presented with no more than two missing teeth, other than third molars, teeth extracted for orthodontic therapy and teeth lost as

a result of extra-oral trauma. Patients also presented with a PD≥6 mm on five to nine interproximal sites. At least two of the qualifying interproximal sites must occur in different quadrants. Gingival inflammation (as exemplified by bleeding on probing) was present in at least two quadrants. Full mouth radiographs must disclose less than four interproximal sites with ≥50% bone loss. Radiographic total mouth mean bone loss must be less than 25%. There is no specifications for CAL in this classification.

Severe Periodontitis: Patients presented with of ≥10 interproximal sites that measure ≥7 mm, with PD of ≥7 mm occurring on at least eight teeth. CAL measured ≥5 mm on ≥11 sites. Full mouth radiographs taken within the last three years showed ≥7 interproximal sites with ≥50% bone loss on radiographs with a total mouth mean bone loss greater than 30%.

Statistical Analysis

χ^2 analysis was used. The Odds Ratio (relative risk) is calculated from a 2×2 contingency table as described by Woolf, 1955.

PCR Amplification and Restriction Enzyme Digestion Protocols for Selected Alleles

IL-1A

The single base variation (C/T) polymorphism at IL-1A base -889 was identified as follows:

SCREENING: PCR amplification of genomic templates. One mismatch inserted in a primer to complete an *Nco*I site if C is available at -889

PRIMERS: The following primers were produced in an ABI DNA synthesizer based on the genomic sequences (Furutani et al., 1986; GENBANK X03833).

5' TGT TCT ACC ACC TGA ACT AGG C 3'
(-967/-945) (SEQ ID No:1)
5' TTA CAT ATG AGC CTT CCA TG 3'
(-888/-869) (SEQ ID No:2)

PCR CONDITIONS:
[96°C (1 minute)] 1 cycle;
[94°C (1 minute), 46°C (1 minute), 72°C (1 minute)] 40 cycles;
[72°C (4 minutes)] 1 cycle.

RESTRICTION ENZYME DIGESTION: Digestion was with *Nco*I at 37°C, for 8 hours. Sizing was by 8% PAGE or 2% agarose gels.

PREDICTED RESULTS FROM DIGESTION:
Allele 1 (C) *Nco*I digestion of PCR products of allele 1 will yield 83 and 16 basepair (bp) fragments.
Allele 2 (T) *Nco*I digestion of PCR products of allele 2 will be ineffective and yield a 99 basepair (bp) product.

IL-1B (*Ava*I)

The single base variation (C/T) polymorphism at IL-1B base -511 was identified as follows:

SCREENING: PCR amplification of genomic templates. The single base variation completes an *Ava*I site on allele 1 (C), a *Bsu*36I site on allele 2 (T).

PRIMERS: The following primers were produced in an ABI DNA synthesizer based on the genomic sequences (Clark et al., 1986; GENBANK X04500).

5' TGG CAT TGA TCT GGT TCA TC 3'
(-702/-682) (SEQ ID No:3)
5' GTT TAG GAA TCT TCC CAC TT 3'
(-417/-397) (SEQ ID No:4)

PCR CONDITIONS:

[95°C (2 minuteS)] 1 cycle;

[95°C (1 minute), 53°C (1 minute), 74°C (1 minute)] 35 cycles;

[74°C (4 minutes)] 1 cycle.

RESTRICTION ENZYME DIGESTION: Digestion was at 37°C, for 8 hours. Sizing was by 8% PAGE.

PREDICTED RESULTS FROM DIGESTION:

Allele 1 (C) *Ava*I digestion of PCR products of allele 1 will yield 190 and 114 bp fragments. *Bsu*36I digestion of PCR products of allele 1 will be ineffective and yield a 304 bp product.

Allele 2 (T) *Ava*I digestion of PCR products of allele 2 will be ineffective and yield a 304 bp product. *Bsu*36I digestion of PCR products of allele 2 will yield 190 and 114 bp fragments.

IL-1B (*Taq*I)

The single base variation (C/T) polymorphism at IL-1B base +3953 was identified as follows:

SCREENING: PCR amplification of genomic templates. One mismatch was inserted in a primer to complete a *Taq*I site as a positive control. Polymorphic *Taq*I site is native.

PRIMERS: The following primers were produced in an ABI DNA synthesizer based on the genomic sequences (Clark et al., 1986; GENBANK X04500).

5' CTC AGG TGT CCT CGA AGA AAT CAA A 3'
(+3844/+3868) (SEQ ID No:5)

5' GCT TTT TTG CTG TGA GTC CCG 3'
(+4017/+4037) (SEQ ID No:6)

PCR CONDITIONS:
[95°C (2 minutes)] 1 cycle;
[95°C(1 minute), 67.5°C (1 minute), 74°C (1 minute)] 38 cycles;
[72°C (8 minutes)] 1 cycle.

RESTRICTION ENZYME DIGESTION: Digestion was at 60°C, for 8 hours. Sizing was by 8% PAGE.

PREDICTED RESULTS FROM DIGESTION:
Allele 1 (C) *Taq*I digestion of PCR products of allele 1 will yield 12, 85 and 97 bp fragments.
Allele 2 (T) *Taq*I digestion of PCR products of allele 2 will yield 12 and 182 bp fragments.

RESULTS

Adults, smokers and nonsmokers, were screened for periodontal disease severity using a consensus clinical criteria as described herein above. The data are shown in Table 1.

TABLE 1

Group	H		M		S	
N	49		42		42	
	mean	S.D.	mean	S.D.	mean	S.D.
BOP *	10.44	7.77	20.84	10.91	26.37	13.63
PD	2.84	0.49	3.85	0.3	4.31	0.46
CAL	2.68	0.89	4.31	0.5	8.66	1.33
#>49%	0	0	0.48	0.67	14.8	7.6
%bl	11.8	2	22.4	2.6	41.8	8.3

Abbreviations used in Tables: PD (pocket depth), BOP (bleeding on probing), CAL (clinical attachment loss), #>49% (number of sites where bone loss is greater than 49%), %bl (percent bone loss), S.D. (standard deviation); H=healthy, M=mild/moderate, S=severe,

* indicates significance at least at 95% confidence level.

In Table 2 the clinical data is displayed and compared between smokers and nonsmokers. Note that there is a significant difference in the overall clinical disease state between smokers and nonsmokers.

TABLE 2

Smoke	No		Yes		p	
N	100		36			
	mean	S.D.	mean	S.D.		
BOP	17.4	11.9	22.85	0.14	0.042	*
PD	3.42	0.62	3.97	0.78	4E-04	**
CAL	3.98	1.61	5.7	2.08	1E-04	**
N	98		35			
#>49%	2.43	5.82	11.59	9.88	1E-04	**
%bl	20.53	10.83	36.76	13.04	1E-04	**

Abbreviations as in Table 1.

Table 3 summarizes and compares the clinical findings for IL-1A alleles 1 and 2. Allelic genotype for each gene is indicated by the paired numbers, i.e. 1/1 indicates homozygosity for allele 1, 1/2 indicates heterozygous for alleles 1 and 2, etc. Where the genotype is given as allele 2 this indicates at least one copy of the allele is present. The analysis is done with the non-smokers. As the data in Table 2 indicates, the smokers as a group had severe disease such that they are not included in the analysis for genetic predisposition. The data in Table 3 shows there is a significant association of severe clinical disease for the carriers of IL-1A allele 2 particularly for percent bone loss, CAL

and PD. The population analyzed included all disease groups for nonsmokers.

TABLE 3

IL-1A	**11**		**12 or 22**			
N	44		54			
	mean	S.D.	mean	S.D.	p	
BOP	16.7	12.7	18	11.6	0.598	
PD	3.28	0.65	3.36	0.58	0.58	
CAL	3.62	1.48	4.3	1.56	0.036	*
#>49%	1.48	4.83	3.2	6.45	0.133	
%bl	17.41	8.77	23.19	11.36	0.006	**

Abbreviations as in Table 1.

In Table 4, the same analysis is performed for IL-1B(*Taq*I) alleles 1 and 2.

TABLE 4

IL-1B (*Taq*I)	**11**		**12 or 22**			
N	51		47			
	mean	S.D.	mean	S.D.	p	
BOP	18.3	12.1	18.6	12	0.341	
PD	3.35	0.56	3.5	0.67	0.234	
CAL	3.82	1.44	4.18	1.79	0.278	
#>49%	1.73	6.07	3.18	6.5	0.218	
%bl	18	9.14	22.32	11.9	0.123	

Abbreviations as in Table 1.

In Table 5, the same analysis is presented for patients who have (+) the genotype IL-1A allele 2 plus IL-1B (*Taq*I) allele 2 versus those who do not (-). More specifically, the - genotype is IL-1A(1/1) plus IL-1B(*Taq*I) (1/1 or 1/2 or 2/2) OR IL-1A (1/2 or 2/2) plus IL-1B(*Taq*I)(1/1). The + genotype is IL-1A (1/2 or 2/2) plus IL-1B(*Taq*I)(1/2 or 2/2).

TABLE 5

Genotype	-		+			
N	63		35			
	mean	S.D.	mean	S.D.	p	
BOP	16.2	12.0	19.5	11.9	0.194	
PD	3.32	0.59	3.62	0.64	0.023	*
CAL	3.7	1.41	4.52	1.83	0.026	*
#>49%	1.43	4.60	4.22	7.26	0.044	*
%bl	18.13	8.74	25.03	12.3	0.005	**

Abbreviations as in Table 1.

The allelic distribution for IL-1A and IL-1B (*Taq*I) according to patient disease severity were determined and are shown in Table 6.

Table 6

ALL SUBJECTS

Patient Genotype		Disease Severity Distribution*		
IL-1A	IL-1B (*Taq*I)	Healthy	Mild-Mod Disease	Severe Disease
1/1	1/1,1/2, 2/2	30 61.2%	16 38.1%	19 44.2%
1/2, 2/2	1/1	8 16.3%	10 23.8%	8 18.6%
1/2, 2/2	1/2, 2/2	11 22.4%	16 38.1%	15 35.7%
		49 100%	42 100%	42 100%

* Distribution is given in both numbers of patients in each category and percent of patients for that disease category.

In Table 7, the results for nonsmokers for IL-1A and IL-1B(*Taq*I) is presented. Of patients with severe disease, 64.7% had a genotype of IL-1A 1/2 or 2/2 and IL-1B(*Taq*I) 1/2 or 2/2 indicating that the presence of

allele 2, either in a heterozygous or homozygous genotype, leads to severe disease susceptibility.

Table 7

NONSMOKERS

Patient Genotype		Disease Severity Distribution		
IL-1A	IL-1B (*Taq*I)	Healthy	Mild-Mod Disease	Severe Disease
1/1	1/1,1/2, 2/2	27 61.4%	13 35.2%	4 23.5%
1/2, 2/2	1/1	7 15.9%	10 27.0%	2 11.8%
1/2, 2/2	1/2, 2/2	10 22.7%	14 37.8%	11 64.7%
		44 100%	37 100%	17 100%

An odds ratio (approximate relative risk) was derived for the association between allelic polymorphism pattern (genotype) at IL-1A allele 2 and IL-1B (*Taq*I) allele 2 and development of disease and/or its severity. The Odds Ratio is calculated by using a Contingency Table as shown in Table 8. The following formula: (A × D)/(C × B) is used to calculate the Odds Ratio (Woolf, 1955).

Table 8
Sample Contingency Table

Genotype of Interest	Phenotype 1	Phenotype 2
Present	A	B
Absent	C	D

As shown in Table 9, patients who are smokers or who have the genotype IL-1A allele 2 plus IL-1B(*Taq*I) allele 2 (+ genotype) are more likely than those who do not have this genotype to have severe disease; they have an Odds Ratio of 10.06:1. Among nonsmokers only (Table

10) the Odds Ratio is 4.3:1 for those patients with the genotype IL-1A allele 2 plus IL-1B(*Taq*I) allele 2.

Table 9
Odds Ratio In All Subjects

Smoker OR Genotype: IL-1A allele 2 plus IL-1B(*Taq*I) allele 2	Severe Disease	Healthy or Mild-Mod Disease
PRESENT	36	34
Absent	6	57

OR = 10.06 (3.84-26.35)

χ^2 = 26.95 ($p<0.0001$)

Table 10

Odds Ratio In Nonsmokers

Genotype: IL-1A allele 2 plus IL-1B(*Taq*I) allele 2	Severe Disease	Healthy or Mild-Mod Disease
PRESENT	11	24
Absent	6	57

OR = 4.3

χ^2 = 7.53 ($p=0.006$)

The clinical data for the smoker or target genotype of IL-1A allele 2 plus IL-1B(*Taq*I) allele 2 (+ genotype) is shown in Table 11.

The allelic distribution for IL-1A and IL-1B (*Ava*I) were determined for Nonsmokers (n=100) and is presented in Table 12. Of patients with severe disease, 36.8% had a genotype of IL-1A 1/2 or 2/2 and IL-1B(*Ava*I) 1/2 or 2/2.

TABLE 11

N	63		70			
	mean	S.D.	mean	S.D.		
BOP	16.24	12.01	21.01	12.98	0.0617	**
PD	8.32	0.59	3.79	0.73	0.0008	**
CAL	3.7	1.41	5.12	2.05	0.0001	**
#>49%	1.43	4.6	7.86	9.24	0.00001	**
% bl	18.13	8.74	30.32	13.68	0.00001	**

Abbreviations as in Table 1.

Table 12

NONSMOKERS

Patient Genotype		Disease Severity Distribution		
IL-1A	IL-1B (*AvaI*)	Healthy	Mild-Mod Disease	Severe Disease
1/1	1/1,1/2, 2/2	27 61.4%	13 35.2%	4 21.1%
1/2, 2/2	1/1	11 25.0%	10 27.0%	8 42.1%
1/2, 2/2	1/2, 2/2	6 13.6%	14 37.8%	**7** **36.8%**
		44 100%	37 100%	19 100%

Among nonsmokers only, the Odds Ratio is 0.85 for those patients with the genotype IL-1A allele 2 plus IL-1B(*AvaI*) allele 2 (Table 13). This genetic combination shows no association with periodontal disease severity.

Table 13
Odds Ratio In Nonsmokers

Genotype: IL-1A allele 2 plus IL-1B(*AvaI*) allele 2	Severe Disease	Healthy or Mild-Mod Disease
PRESENT	10	46
Absent	9	35

The data presented show that of those subjects with severe disease 86.0% were either current smokers or had the target genotype IL-1A allele 2 plus IL-1B(*Taq*I) allele 2. Of the subjects who were neither current smokers nor had the target genotype, 90.5% did not have severe disease. Of the subjects who were either current smokers or had the target genotype, 52.1% had severe disease independent of any other risk factor.

The present invention therefore provides a method of identifying patients at risk for severe periodontal disease to allow early treatment.

Throughout this application various publications and patents are referenced. Full citations for the referenced publications and patents not included herein above are listed below. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

The invention has been described in an illustrative manner, and it is to be understood that the terminology which has been used is intended to be in the nature of words of description rather than of limitation.

Obviously, many modifications and variations of the present invention are possible in light of the above teachings. It is, therefore, to be understood that within the scope of the appended claims, the invention may be practiced otherwise than as specifically described.

REFERENCES

Blakemore et al., "Interleukin-1 receptor antagonist gene polymorphism as a severity factor in systemic lupus erythematosus" *Arthritis and Rheumatism* 37(9):1380-1385 (1994).

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de Giovine et al., "Single base polymorphism at -511 in the human interleukin-1β gene (IL1β)" *Human Molecular Genetics* 1, No. 6:450 (1992).

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McDowell et al., "A genetic association between juvenile rheumatoid arthritis and a novel interleukin-1 alpha polymorphism" *Arthritis & Rheumatism* (in press 1995).

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Michalowicz et al., "Periodontal findings in adult twins" *J Periodontol* 62:293-299 (1991)

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Verjans et al., "Polymorphism of the tumor necrosis factor region in relation to disease: An overview" *Rheum Dis Clin North Am* 18:177-186 (1992).

Wilson et al., "Single base polymorphism in the human Tumor Necrosis Factor alpha (TNFα) gene detectable by *Nco*1 restriction of PCR product" *Human Molecular Genetics* 1, No. 5:353 (1992).

Woolf, B., "Estimating the relationship between blood groups and disease" *Annals of Human Genetics* 19:251-253 (1955).

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Kornman, Kenneth S.
Duff, Gordon W.

(ii) TITLE OF INVENTION: Detecting Genetic Predisposition to Periodontal Disease

(iii) NUMBER OF SEQUENCES: 6

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TGTTCTACCA CCTGAACTAG GC 22

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TTACATATGA GCCTTCCATG 20

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TGGCATTGAT CTGGTTCATC 20

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GTTTAGGAAT CTTCCCACTT 20

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CTCAGGTGTC CTCGAAGAAA TCAAA 25

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GCTTTTTTGC TGTGAGTCCC G 21

CLAIMS

1. A kit for the identification of a patient's genetic polymorphism pattern at IL-1A and IL-1B associated with increased periodontal disease severity including

DNA sample collecting means, and

means for determining a genetic polymorphism pattern and to compare the pattern to control samples of known disease severity to determine a patient's susceptibility to periodontal disease severity.

2. The kit as set forth in claim 1 wherein the control samples are ethnically matched control samples of known disease severity.

3. The kit as set forth in claim 1 wherein the means for determining genetic polymorphism pattern include amplification of DNA target sequences using the polymerase chain reaction (PCR) wherein the PCR primers used are

5' TGT TCT ACC ACC TGA ACT AGG C 3' (SEQ ID No:1);

5' TTA CAT ATG AGC CTT CCA TG 3' (SEQ ID No:2);

5' TGG CAT TGA TCT GGT TCA TC 3' (SEQ ID No:3);

5' GTT TAG GAA TCT TCC CAC TT 3' (SEQ ID No:4);

5' CTC AGG TGT CCT CGA AGA AAT CAA A 3' (SEQ ID No:5); and

5' GCT TTT TTG CTG TGA GTC CCG 3' (SEQ ID No:6).

4. The kit as set forth in claim 1 wherein the means for determining genetic polymorphism patterns include restriction enzyme digestion with restriction enzymes *Nco*I, *Taq*I, *Ava*I and *Bsu*36I.

5. A method for predicting increased periodontal disease severity including the steps of

isolating genomic DNA from a patient,

identifying in the isolated genomic DNA from a patient a genetic polymorphism pattern for interleukins IL-1α and IL-1β,

comparing the identified pattern to control patterns of known disease severity, and

identifying patients expressing a genetic polymorphism pattern associated with increased periodontal disease severity.

6. The method as set forth in claim 5 wherein the control samples are ethnically matched control samples of known disease severity.

7. The method as set forth in claim 5 wherein said step for identifying in the DNA a genetic polymorphism pattern for IL-1A and IL-1B includes amplification of target DNA sequences using the polymerase chain reaction (PCR) wherein the PCR primers used are:

5' TGT TCT ACC ACC TGA ACT AGG C 3' (SEQ ID No:1);
5' TTA CAT ATG AGC CTT CCA TG 3' (SEQ ID No:2);
5' TGG CAT TGA TCT GGT TCA TC 3' (SEQ ID No:3);
5' GTT TAG GAA TCT TCC CAC TT 3' (SEQ ID No:4);
5' CTC AGG TGT CCT CGA AGA AAT CAA A 3' (SEQ ID No:5);
and
5' GCT TTT TTG CTG TGA GTC CCG 3' (SEQ ID No:6).

8. The method as set forth in claim 5 wherein said step for identifying in the DNA a genetic polymorphism pattern for IL-1A and IL-1B includes restriction enzyme digestion with restriction enzymes *Nco*I, *Taq*I, *Ava*I and *Bsu*36I.

9. The method as set forth in claim 5 wherein the DNA genetic polymorphism pattern associated with disease severity is IL-1A allele 2 plus IL-1B(*Taq*I) allele 2.

10. The method as set forth in claim 5 wherein the DNA genetic polymorphism pattern associated with disease severity is the presence of at least one copy of IL-1A allele 2.

11. A product in the form of a periodontal disease severity report based upon determining a genetic polymorphism pattern for interleukins IL-1α and IL-1β associated with increased periodontal disease severity produced by the steps of

identifying in the isolated genomic DNA from a patient a genetic polymorphism pattern for interleukins IL-1α and IL-1β,

comparing the identified pattern to control patterns of known disease severity,

identifying patients expressing a genetic polymorphism pattern associated with increased periodontal disease severity, and

identifying and preparing a report identifying patients expressing a genetic polymorphism pattern associated with increased periodontal disease severity.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/12455

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C07H 21/04; C12Q 1/68; C12P 19/34
US CL :536/24.31, 24.33; 435/6. 91.2
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/24.31, 24.33; 435/6. 91.2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

NONE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y -- A	POCIOT et al. A TaqI polymorphism in the human interleukin-1β (IL-1β) gene correlates with IL-1β secretion in vitro. European Journal of Clinical Investigation 1992, Vol. 22, pages 396-402, especially page 397 and Figure 1.	1, 2, 4 --- 3, 5-10
A	DI GIOVINE et al. Single base polymorphism at -511 in the human interleukin-1β gene (IL1β). Human Molecular Genetics. September 1992, Vol. 1, No. 6, page 450, see entire document.	1-10

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 10 OCTOBER 1996	Date of mailing of the international search report 07 NOV 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer CARLA MYERS Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/12455

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MANSFIELD et al. Novel genetic association between ulcerative colitis and the anti-inflammatory cytokine interleukin-1 antagonist. Gastroenterology. 1994, Vol. 106, No. 3, pages 637-642, especially Figure 1 and Table 2.	1-10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/12455

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 11
because they relate to subject matter not required to be searched by this Authority, namely:

because the claimed subject matter was drawn to a report in the form of a written document.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.